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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NMRI 84-08	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SYNERGISM BETWEEN PENICILLIN, CLINDAMYCIN, OR METRONIDAZOLE AND GENTAMICIN AGAINST SPECIES OF THE BACTEROIDES MELANINOGENICUS AND BACTEROIDES FRAGILIS GROUPS		5. TYPE OF REPORT & PERIOD COVERED MEDICAL RESEARCH PROGRESS REPORT 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) I. Brook, J.C. Coolbaugh, R.I. Walker, and E. Weiss		8. CONTRACT OR GRANT NUMBER(s) Upjohn 68011 (partially)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Medical Research Institute Bethesda, Maryland 20814		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research & Development Command Bethesda, Maryland 20814		12. REPORT DATE 1984 13. NUMBER OF PAGES 7 (seven)
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Naval Medical Command Department of the Navy Washington, DC 20372		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE AND SALE. DISTRIBUTION UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

REPRINT: Antimicrobial Agents and Chemotherapy, 1984 JAN; 25(1):71-7

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Bacteroides melaninogenicus, Bacteroides fragilis, MICs, penicillin, clindamycin, metronidazole

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1984, p. 71–77
0066-4804/84/010071-07$02.00/0

Vol. 25, No. 1

Synergism Between Penicillin, Clindamycin, or Metronidazole and Gentamicin Against Species of the *Bacteroides melaninogenicus* and *Bacteroides fragilis* Groups

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Received 10 August 1983 Accepted 17 October 1983

Clinical isolates of the *Bacteroides melaninogenicus* and *Bacteroides fragilis* groups were tested for in vitro and in vivo susceptibility to penicillin, clindamycin, and metronidazole, used singly or in combination with gentamicin. The in vitro tests consisted of determinations of minimal inhibitory concentrations (MICs) carried out with or without constant amounts of gentamicin. When used alone, gentamicin had negligible effects on the bacteria but significantly reduced the MICs of penicillin, clindamycin, and metronidazole against 11, 10, and 3, of the 15 strains of the *B. melaninogenicus* group, respectively. The 15 strains of the *B. fragilis* group were all β-lactamase producers and were highly resistant to penicillin or the combination of penicillin and gentamicin. However, gentamicin reduced the MICs of clindamycin and metronidazole against 1 and 7 strains of this group, respectively. The in vivo tests were carried out in mice and consisted of measurements of the effects of the antimicrobial agents on the sizes and bacterial content of abscesses induced by subcutaneous injection of bacterial suspensions. The results of the in vivo tests were generally consistent with those obtained in vitro with strains of the *B. melaninogenicus* group. Synergism between gentamicin and penicillin, clindamycin, or metronidazole was shown in 13, 10, and 3 strains of this group, respectively. In vivo synergism was not clearly demonstrated with the strains of the *B. fragilis* group, possibly because clindamycin and metronidazole used alone were highly efficacious. We suggest that the synergistic effect of gentamicin is due to its increased transport into the bacterial cell in the presence of penicillin and, possibly, other antimicrobial agents. The newly recognized in vitro and in vivo synergism between penicillin and other antimicrobial agents and an aminoglycoside in *B. melaninogenicus* may have clinical implications that deserve to be investigated.

Anaerobic bacteria of the *Bacteroides fragilis* and *Bacteroides melaninogenicus* groups are important clinical pathogens, *B. fragilis* in intra-abdominal abscesses (2, 7), *B. melaninogenicus* in lung and upper respiratory infections (1). Since *Bacteroides* spp. are often recovered from mixed infections with aerobic bacteria, penicillin or a cephalosporin is commonly administered for the treatment of infections due to *B. melaninogenicus* group bacteria, and chloramphenicol, cefoxitin, clindamycin, or metronidazole is given for *B. fragilis* group infections. When enteric gram-negative organisms are suspected in addition to anaerobes, aminoglycosides are also administered.

A number of investigators have been concerned with the possibility of antagonism between antibiotics, which, fortunately, has not been encountered with drugs used against *Bacteroides* spp. (4, 5, 10, 16). A by-product of these studies has been the discovery that some antibiotics act synergistically against *B. fragilis*. For example, Fass et al. (5) reported in vitro synergism between clindamycin and the aminoglycoside gentamicin. These results were confirmed by Okubadejo and Allen (16), who found this combination to be more effective than clindamycin combined with kanamycin. Busch et al. (4) obtained a significant synergistic effect between clindamycin and the antimicrobial agent metronidazole in 13 of 17 strains. Ralph and Amatnieks (18) tested six drugs in combination with metronidazole and found that nalidixic acid, clindamycin, and rifampin had a synergistic effect on some strains. Thadepalli et al. (20) obtained excellent synergistic activity between cefuroxime and penicillin or carbenicillin in two of three strains.

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This work was prompted by the need to extend the above-described observations on drug synergism to other antibacterial combinations and to strains of the *B. melaninogenicus* group. We show that, with many strains, gentamicin, which by itself has a negligible inhibitory effect on *Bacteroides* spp., is very effective in combination with penicillin, clindamycin, or metronidazole in reducing the minimal inhibitory concentrations (MICs) of these antimicrobial agents and in suppressing abscess formation in infected mice.

(The experiments conducted herein were carried out in accordance with the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institutes of Laboratory Resources, National Research Council, Department of Health, Education, and Welfare publication no. (NIH 74-23).)

MATERIALS AND METHODS

Bacteria. All *Bacteroides* strains were recent isolates from clinical specimens obtained from the Children's Hospital, Washington, D.C., or from the Naval Medical Command, National Capital Region, Bethesda, Md. They were identified in this laboratory by standard procedures (8, 19). Of 15 isolates of the *B. melaninogenicus* group, 7 were *Bacteroides intermedius*, 4 were *Bacteroides asaccharolyticus*, and 4 were *B. melaninogenicus*. Of 15 isolates of the *B. fragilis* group, 6 were *B. fragilis* and 3 each were *Bacteroides vulgatus*, *Bacteroides ovatus*, and *Bacteroides thetaiotaomicron* (see Tables 1 and 2). All strains were encapsulated as confirmed by the Hiss staining method (14) and by electron microscopy after staining with ruthenium red (9). Stock suspensions were stored in skim milk at −70°C. For the experiments described here, the bacteria were grown anaer-

obically on blood agar plates with a brain heart infusion base (Difco Laboratories, Detroit, Mich.) for a total of two or three passages after isolation.

Animals. Male Swiss albino mice weighing 20 to 25 g each were obtained from the Naval Medical Research Institute mouse colony. The mice were raised under conventional conditions.

Antimicrobial agents. The following antimicrobial agents, obtained from the indicated sources, were used: penicillin G, E. R. Squibb & Sons, Inc., Princeton, N.J.; clindamycin, The Upjohn Co., Kalamazoo, Mich.; metronidazole, G. D. Searle & Co., Chicago, Ill.; and gentamicin, Schering Corp., Kenilworth, Ill. MICs were determined by the agar dilution method (19) with a series of nine concentrations of each agent. Each experiment was repeated twice. For penicillin G, the concentrations were 12, 6, 3, 1.5, 0.75, 0.2, 0.1, 0.05, and 0.01 μg ml, except that concentrations of as high as 64 μg ml were used in preliminary tests with strains of the *B. fragilis* group. For clindamycin, metronidazole, and gentamicin, twofold dilutions were used from 10 to 0.04, 50 to 0.1, and 200 to 0.8 μg ml, respectively. The in vitro synergistic effect of gentamicin was determined by adding 10 μg of gentamicin per ml to each dilution of the antimicrobial agent to be tested. The effect was considered synergistic if it reduced the MIC of the associated antibacterial agent by fourfold or more.

β-Lactamase activity was determined for all microorganisms by use of a chromogenic cephalosporin substrate (15).

Infection of mice and antimicrobial therapy. Experiments usually consisted of 150 to 200 animals tested simultaneously, with 6 mice per experimental group, except 10 mice were used for determination of the levels of antimicrobial agents in serum and in abscesses. Mice were infected by subcutaneous injection into the right groin of 0.1-ml volumes of suspensions in saline containing 10^9 bacteria per ml. The antimicrobial agents, used singly or mixed with gentamicin, were administered intramuscularly on alternate thighs 2 h after inoculation and at 8-h intervals for 7 days. The amounts administered, in terms of a 20-g mouse per injection, were as follows: penicillin G, 0.67 mg; clindamicin, 0.27 mg; metronidazole, 0.33 mg; and gentamicin, 0.05 mg.

The mice were sacrificed on day 7 by cervical dislocation. The sizes of the abscesses were estimated from measurements by caliper of two perpendicular diameters, corresponding to maximum length and width. The product of these two measurements, expressed as millimeters squared, was approximately proportional to the outer surface of the abscess. For the determination of the bacterial contents of the abscesses, the abscess material was removed aseptically and homogenized in an anaerobic glove box in 1 ml of sterile saline in a ground glass tissue homogenizer. Tenfold serial dilutions of the homogenates were made in sterile saline, and 0.1-ml volumes of each dilution was spread in triplicate on brain heart infusion-enriched blood agar plates. No attempt was made to inactivate the antimicrobial agents in the homogenized abscess material, since a considerable dilution was achieved before plating, especially with the smaller abscesses. Colonies were counted after incubation at 37°C in an anaerobic chamber for 48 h, and the results are presented as $\log_{10}$ of viable bacteria per abscess.

In vivo synergism was defined as a significant reduction ($P < 0.01$) in abscess size associated with addition of gentamicin to the other antimicrobial agent. Statistical analyses were accomplished with the Student *t* test of independent means.

The levels of the antimicrobial agents in sera and abscesses were determined in a separate group of mice by the following methods: penicillin G and clindamycin, agar diffusion assay (12) with *Micrococcus luteus* ATCC 9341 (American Type Culture Collection, Rockville, Md.); metronidazole, high-pressure chromatography (21); gentamicin, agar diffusion assay with *Bacillus subtilis* ATCC 6051.

RESULTS

In vitro susceptibility of *Bacteroides* strains. Table 1 shows the MICs of 15 isolates of the *B. melaninogenicus* group with respect to three antimicrobial agents tested alone or in combination with 10 μg of gentamicin per ml. Although none of the isolates produced β-lactamase, susceptibility to penicillin varied greatly, requiring MICs ranging from 0.01 to 6 μg ml. The MICs for 5 of 6 strains requiring high MICs and several requiring moderate MICs, a total of 11, were significantly reduced by gentamicin. Susceptibility to clindamycin varied somewhat less, with MICs ranging from 0.31 to 2.5 μg ml. Ten of these MICs were reduced by gentamicin. With metronidazole, the MICs were close to the lower concentrations used in the test, 0.2 to 1.6 μg ml, and the MICs of only three strains were reduced by gentamicin. It is possible, however, that the concentrations used were not sufficiently low to detect all possible instances of synergism with strains of the *B. melaninogenicus* and *B. fragilis* groups. Gentamicin by itself had little if any inhibitory effect on strains of either group (Tables 1 and 2).

All 15 strains of the *B. fragilis* group were β-lactamase producers and highly resistant to penicillin (MICs, >64 μg ml) in the presence or absence of gentamicin (data not shown). The MICs of clindamycin and metronidazole alone and in combination with gentamicin are shown in Table 2. The MIC of clindamycin ranged from 0.15 to 2.5 μg ml, but the MIC was reduced by gentamicin for only one strain, from 0.15 to 0.04 μg ml. The MIC of metronidazole ranged from 0.2 to 6.25 μg ml. The MICs for seven strains were reduced to a moderate extent by gentamicin.

In vivo effect of combined antimicrobial therapy. The 30 isolates used in these studies reproducibly elicited abscesses when injected into the groins of mice. Since these strains were encapsulated, no virulence-enhancing factor was required (9). The sizes of these abscesses (see Tables 3 and 4) are measurements of outer surfaces, as described in Materials and Methods. This type of measurement was used in preference to volume because the thickness of the abscesses could not be estimated with accuracy and the abscesses were not firm enough to be dissected out and weighed. Without antimicrobial therapy, the abscesses achieved a mean outer surface size of about 300 mm^2, with relatively small differences among the strains.

The effects of antimicrobial therapy on abscess formation of isolates of the *B. melaninogenicus* group are shown in Table 3. The abscesses induced by 12 of 15 strains were reduced to a significant but relatively moderate extent by penicillin. A substantial further reduction was achieved with a combination of penicillin and gentamicin with these 12 strains and with one which had not been affected by penicillin administered singly. The results (Table 3) agreed reasonably well from those expected from MIC determinations (Table 1). An unexplained discrepancy is the low MICs of penicillin for strain 8 of *B. asaccharolyticus* and the lack of in vivo efficacy. Clindamycin greatly reduced the sizes of all abscesses, and an even greater reduction was achieved with nine strains when the drug was combined with gentamicin. These results are in good agreement with those shown in Table 1. Metronidazole proved to be efficacious against all

TABLE 1. MICs of penicillin, clindamycin, and metronidazole alone and in combination with gentamicin for isolates of the *B. melaninogenicus* group

Isolate[a]	MIC (μg/ml) of[b]: Penicillin G		Clindamycin		Metronidazole		Gentamicin alone
	−	+	−	+	−	+	
B. intermedius							
7	6	0.1[c]	0.62	0.62	0.4	0.4	100
12	0.01	0.01	0.62	0.62	0.8	0.8	200
37	0.1	0.01[c]	0.62	0.04[c]	1.6	1.6	100
54	3	0.01[c]	2.5	0.62[c]	0.8	0.8	100
55	6	0.01[c]	2.5	0.62[c]	0.4	0.2	200
397	1.5	0.01[c]	1.25	0.62	1.6	0.2[c]	100
509	1.5	0.01[c]	2.5	0.04[c]	1.6	0.2[c]	200
B. asaccharolyticus							
8	0.01	0.01	1.25	0.04[c]	0.8	0.8	200
114	1.5	0.05[c]	1.25	0.04[c]	0.4	0.4	200
221	6	0.01[c]	1.25	0.62	0.8	0.4	100
281	6	0.01[c]	0.31	0.08[c]	0.4	0.4	100
B. melaninogenicus							
172	1.5	0.01[c]	0.31	0.31	0.8	0.2[c]	200
292	1.5	0.01[c]	0.31	0.04[c]	0.8	0.8	200
375	0.1	0.1	1.25	0.31[c]	0.4	0.4	100
446	6	3	0.62	0.04[c]	0.2	0.2	100
Synergistic combinations/total		11/15		10/15		3/15	

[a] Naval Medical Research Institute number.
[b] −, Without gentamicin; +, with gentamicin (10 μg/ml).
[c] Synergistic effect as defined in the text.

TABLE 2. MICs of clindamycin and metronidazole alone and in combination with gentamicin for isolates of the *B. fragilis* group

Isolate[a]	MIC (μg/ml) of[b]: Clindamycin		Metronidazole		Gentamicin alone
	−	+	−	+	
B. fragilis					
13	0.31	0.31	0.8	0.2[c]	200
21	0.15	0.08	0.8	0.2[c]	200
38	0.15	0.04[c]	0.4	0.2	100
43	0.31	0.31	0.8	0.2[c]	200
52	1.25	0.62	0.2	0.2	200
181	1.25	1.25	3.1	3.1	200
B. vulgatus					
200	0.62	1.25	0.8	0.2[c]	100
360	0.62	0.62	0.8	0.2[c]	200
583	1.25	0.62	0.8	0.2[c]	200
B. ovatus					
22	0.62	0.62	1.6	1.6	200
105	0.31	0.31	0.8	0.8	200
234	0.15	0.08	0.4	0.2	200
B. thetaiotaomicron					
27	1.25	0.62	6.2	1.6[c]	100
85	0.62	0.62	3.1	1.6	200
176	2.5	2.5	3.1	1.6	200
Synergistic combinations/total		1/15		7/14	

[a] Naval Medical Research Institute number.
[b] −, Without gentamicin; +, with gentamicin (10 μg/ml).
[c] Synergistic effect as defined in the text.

strains. However, synergism with gentamicin, apparent in four strains, did not correlate well with in vitro synergism. Gentamicin administered by itself did not result in a reduction in the sizes of the abscesses, but surprisingly, in some cases elicited a significant increase.

As expected, none of the abscesses elicited by the 15 strains of the *B. fragilis* group were affected by penicillin or a combination of penicillin and gentamicin (data not shown). Similarly, gentamicin was without effect, except that it elicited an increase in the sizes of abscesses induced by one strain (Table 4). As expected from the data shown in Table 2, clindamycin and metronidazole were efficacious against all strains, but synergism with gentamicin was not clearly demonstrated in vivo.

The viable counts of the abscesses described in Tables 3 and 4 are shown in Tables 5 and 6. With strains of the *B. melaninogenicus* group (Table 5) the number of CFU in untreated mice were relatively uniform, with means ranging from $10^{8.8}$ to $10^{10.4}$ per abscess. Penicillin administered singly reduced the number of CFU of all strains, even in cases in which a therapeutic effect was not demonstrated. The reduction varied from about 2 to 6 logs. When penicillin was administered in combination with gentamicin, the viable counts were generally reduced to 10^2 or less. The four exceptions ($10^{4.2}$ to $10^{6.4}$) included the two instances of abscesses which were not reduced in size by chemotherapy. Clindamycin and metronidazole administered singly greatly reduced the number of CFU, with relatively few means exceeding 10^3 per abscess. Because of the relatively small number of CFU, a further reduction by combination chemotherapy is apparent in only a few cases. Gentamicin administered singly did not reduce the number of CFU. The possibil-

TABLE 3. Impacts of treatment with penicillin, clindamycin, and metronidazole alone and in combination with gentamicin on abscess sizes in mice infected with isolates of the *B. melaninogenicus* group

Isolate[a]	Mean ± SD abscess size (mm²) after treatment with following drug dose (mg/kg per day)[b]							
	None (untreated mice)	Penicillin G (100)		Clindamycin (40)		Metronidazole (50)		Gentamicin alone (7.5)
		−	+	−	+	−	+	
B. intermedius								
7	288 ± 26	146 ± 12[c]	12 ± 14[d]	18 ± 12[c]	24 ± 6	22 ± 8[c]	16 ± 7	362 ± 34
12	314 ± 28	118 ± 14[c]	18 ± 12[d]	24 ± 12[c]	19 ± 13	32 ± 12[c]	2 ± 4[d]	284 ± 17
37	317 ± 14	128 ± 12[c]	38 ± 9[d]	26 ± 3[c]	1 ± 1[d]	56 ± 11[c]	4 ± 3[d]	342 ± 39
54	296 ± 24	94 ± 16[c]	24 ± 8[d]	36 ± 8[c]	4 ± 6[d]	26 ± 7[c]	30 ± 18	308 ± 28
55	274 ± 32	108 ± 22[c]	40 ± 6[d]	24 ± 6[c]	2 ± 4[d]	18 ± 8[c]	14 ± 17	342 ± 52
397	328 ± 32	131 ± 16[c]	34 ± 14[d]	18 ± 10[c]	18 ± 11	14 ± 10[c]	24 ± 8	346 ± 48
509	342 ± 42	148 ± 24[c]	28 ± 18[d]	30 ± 16[c]	6 ± 2[d]	24 ± 12[c]	3 ± 2[d]	328 ± 30
B. asaccharolyticus								
8	318 ± 28	294 ± 24	242 ± 15	16 ± 12[c]	2 ± 1	22 ± 12[c]	16 ± 6	324 ± 16
114	300 ± 18	277 ± 17	24 ± 6[d]	28 ± 0[c]	2 ± 1[d]	63 ± 10[c]	5 ± 6[d]	528 ± 19[c]
221	332 ± 41	142 ± 22[c]	51 ± 12[d]	12 ± 6[c]	14 ± 6	15 ± 8[c]	18 ± 8	482 ± 62[c]
281	285 ± 38	102 ± 16[c]	24 ± 16[d]	30 ± 4[c]	1 ± 0[d]	21 ± 3[c]	13 ± 0	308 ± 42
B. melaninogenicus								
172	342 ± 30	130 ± 21[c]	22 ± 16[d]	18 ± 12[c]	24 ± 10	32 ± 7[c]	18 ± 4	328 ± 37
292	330 ± 27	132 ± 24[c]	14 ± 3[d]	33 ± 4[c]	1 ± 1[d]	28 ± 7[c]	28 ± 13	498 ± 26[c]
375	352 ± 24	142 ± 16[c]	16 ± 8[d]	28 ± 20[c]	6 ± 3	24 ± 5[c]	40 ± 19	368 ± 40
446	284 ± 10	265 ± 48	194 ± 42	32 ± 18[c]	2 ± 4[d]	18 ± 12[c]	22 ± 13	412 ± 52[c]

[a] The mice were infected and treated as described in Materials and Methods. The abscess sizes, determined on day 7 postinfection, are expressed as the products of two surface dimensions (millimeters squared) and are presented as the means ± standard deviations of six mice in each group. −, Without gentamicin; +, with gentamicin (7.5 mg/kg per day).

[c] Significant reduction ($P < 0.01$) of abscess size by a single antimicrobial agent (without gentamicin).

[d] Significant synergistic effect ($P < 0.01$) with gentamicin.

[e] Significant increase ($P < 0.01$) of abscess size.

ity that in some cases it may have enhanced them cannot be excluded.

With strains of the *B. fragilis* group (Table 6), the number of CFU in the abscesses of untreated mice were also relatively uniform, from $10^{8.6}$ to $10^{10.8}$, and the counts in the gentamicin-treated mice were approximately the same. Clindamycin had a variable effect on the number of CFU, reducing them by about 2 to 8 logs. A further reduction by this drug in combination with gentamicin is not apparent. The reduction achieved by metronidazole was somewhat higher, from about 3 to 9.5 logs. In combination with gentamicin, metronidazole significantly reduced the number of CFU in only one instance (*B. fragilis* 13). The abscesses elicited by this strain and this drug combination were also reduced to a small but not highly significant extent (Table 4).

The concentrations of the antimicrobial agents in sera and in the abscesses of mice were checked with only two strains (*B. asaccharolyticus* 114 and *B. fragilis* 13) and only on day 7 after infection (Table 7). It is obvious that sufficient levels were achieved in both locations to inhibit the susceptible strains. It is interesting to note that the penicillin concentrations in the abscesses were considerably lower in mice infected with the *B. fragilis* strains than in those infected with *B. asaccharolyticus*, possibly because the *B. fragilis* strains but not the *B. asaccharolyticus* strains contained β-lactamase which destroyed penicillin.

DISCUSSION

In this study, 30 strains of *Bacteroides* spp. were tested for their susceptibilities to four antimicrobial agents plus three antimicrobial agent combinations by three criteria: MIC, reduction in sizes of abscesses elicited in mice, and bacterial content in these abscesses. Often, but not always, low MICs correlated well with small abscess sizes and low bacterial contents. Some of the more obvious discrepancies have already been noted. In other cases, although the general trend was the same, there were large differences in the bacterial content of abscesses of approximately equal size (compare, for example, the effect of clindamycin in Tables 4 and 6). These discrepancies and qualitative differences may in some cases be due to imperfections of the animal model and methodology used. However, the mice appeared to have tolerated well the multiple injections of the antimicrobial agents, and our limited test of antimicrobial agent content in sera and abscesses indicated that the dosage was adequate. Undoubtedly, unrecognized variations in the physiological properties of the strains played an important role. Thus, both in vitro and in vivo results must be taken into consideration before any conclusions are derived from this study.

Previous investigations of the effect on *Bacteroides* spp. of antibiotics and antibiotic combinations were primarily concerned with *B. fragilis* (4–6, 10–13, 16, 18, 20, 22). There is general agreement that gentamicin by itself is relatively ineffective (6). The exacerbation of some of the abscesses seen in this study was possibly due to interference with the defense mechanism of the hosts (unpublished observations).

Metronidazole has been recognized as one of the most effective antimicrobial agents, consistently inhibitory and bactericidal at achievable in vivo concentrations (22). Because of this finding, this agent has been most frequently studied in combination with other antibiotics, such as clindamycin (4, 18) and spiramycin (11), which proved to be synergistic. The combination of clindamycin and gentamicin has been found to be synergistic by some (5, 16) but not by all investigators (10).

TABLE 4. Impacts of treatment with clindamycin and metronidazole alone and in combination with gentamicin on abscess sizes in mice infected with isolates of the *B. fragilis* group

Isolate	Mean ± SD abscess size (mm²) after treatment with following drug dose (mg kg per day)[a]					
	None (untreated mice)	Clindamycin (40)		Metronidazole (50)		Gentamicin alone (7.5)
		−	+	−	+	
B. fragilis						
13	336 ± 29	80 ± 11[b]	69 ± 13	44 ± 7[b]	25 ± 9	381 ± 36
21	348 ± 34	65 ± 12[b]	48 ± 21	42 ± 8[b]	17 ± 12	380 ± 21
38	296 ± 42	82 ± 14[b]	64 ± 14	38 ± 12[b]	28 ± 14	314 ± 36
43	318 ± 48	32 ± 21[b]	42 ± 12	18 ± 11[b]	31 ± 13	362 ± 42
52	352 ± 28	16 ± 8[b]	28 ± 9	12 ± 10[b]	16 ± 15	342 ± 18
181	364 ± 26	28 ± 7[b]	34 ± 12	21 ± 12[b]	17 ± 9	319 ± 53
B. vulgatus						
200	312 ± 28	46 ± 6[b]	41 ± 18	34 ± 12[b]	31 ± 20	284 ± 45
360	342 ± 20	58 ± 8[b]	63 ± 10	66 ± 12[b]	69 ± 10	326 ± 20
583	278 ± 18	62 ± 13[b]	54 ± 21	56 ± 18[b]	42 ± 7	262 ± 3
B. ovatus						
22	300 ± 18	96 ± 8[b]	96 ± 15	37 ± 5[b]	51 ± 11	305 ± 25
105	316 ± 16	82 ± 21[b]	72 ± 15	64 ± 7[b]	51 ± 18	312 ± 26
234	348 ± 24	16 ± 12[b]	19 ± 11	22 ± 11[b]	23 ± 13	315 ± 38
B. thetaiotaomicron						
27	300 ± 18	86 ± 10[b]	96 ± 15	24 ± 0[b]	18 ± 5	406 ± 17[c]
85	298 ± 34	48 ± 15[b]	54 ± 21	40 ± 8[b]	42 ± 15	316 ± 44
176	348 ± 28	62 ± 17[b]	59 ± 18	32 ± 2[b]	22 ± 13	308 ± 32

[a] The mice were infected as described in Materials and Methods. The abscess sizes, determined on day 7 postinfection, are expressed as the products of two surface dimensions (millimeters squared) and are presented as the means ± standard deviations of six mice in each group. −, Without gentamicin; +, with gentamicin (7.5 mg kg per day).

[b] Significant reduction ($P < 0.01$) of abscess size by a single antimicrobial agent (without gentamicin).

[c] Significant increase ($P < 0.01$) of abscess size.

Our in vitro results with strains of the *B. fragilis* group are not surprising. The fact that in vitro synergism between clindamycin or metronidazole and gentamicin was not clearly reflected in in vivo abscess reduction might be attributed to the efficacy of clindamycin and metronidazole used singly.

More significant are our results of in vitro and in vivo synergism between penicillin and gentamicin against *B.*

TABLE 5. Impacts of treatment with penicillin, clindamycin, and metronidazole alone and in combination with gentamicin on CFU in abscesses of mice infected with isolates of the *B. melaninogenicus* group

Isolate	Mean ± SD $\log_{10}$ CFU after treatment with following drug dose (mg kg per day)[a]							
	None (untreated mice)	Penicillin G (100)		Clindamycin (40)		Metronidazole (50)		Gentamicin alone (7.5)
		−	+	−	+	−	+	
B. intermedius								
7	9.7 ± 0.6	6.8 ± 0.3	1.9 ± 0.6	1.2 ± 0.8	<1.0	<1.0	<1.0	9.7 ± 0.6
12	8.8 ± 1.2	6.2 ± 0.4	4.2 ± 0.3	1.4 ± 0.6	<1.0	2.2 ± 0.4	<1.0	9.2 ± 0.8
33	9.6 ± 0.8	5.4 ± 0.6	2.0 ± 0.2	1.8 ± 0.4	<1.0	1.4 ± 0.8	1.2 ± 0.6	9.4 ± 0.6
54	9.8 ± 0.4	4.4 ± 0.4	1.2 ± 1.4	<1.0	1.2 ± 0.4	2.0 ± 1.1	2.2 ± 0.2	9.6 ± 0.8
55	10.2 ± 0.8	4.4 ± 0.4	<1.0	2.4 ± 0.6	<1.0	2.4 ± 0.4	1.8 ± 0.2	11.8 ± 1.4
397	8.8 ± 0.5	5.2 ± 0.8	1.4 ± 0.6	1.8 ± 1.0	<1.0	<1.0	2.1 ± 0	10.2 ± 1.0
509	9.4 ± 0.8	7.3 ± 1.4	1.3 ± 1.1	2.8 ± 0.4	1.2 ± 0.2	2.2 ± 0.4	1.8 ± 0.4	9.8 ± 1.2
B. asaccharolyticus								
8	9.6 ± 0.5	5.4 ± 1.2	4.8 ± 1.2	2.6 ± 0.6	<1.0	1.6 ± 0.4	1.8 ± 0.4	10.8 ± 1.2
114	10.0 ± 0.5	7.8 ± 0.6	1.5 ± 0.9	1.3 ± 1.0	<1.0	2.3 ± 0.7	1.7 ± 0.4	11.1 ± 0.4
221	9.8 ± 0.9	6.5 ± 0.8	1.4 ± 0.4	1.8 ± 0.4	1.2 ± 0.4	2.1 ± 0.8	2.5 ± 0.6	11.2 ± 1.2
281	10.2 ± 0.4	8.6 ± 0.9	1.5 ± 1.0	2.6 ± 0.6	1.2 ± 0.4	1.8 ± 0.6	<1.0	9.6 ± 0.8
B. melaninogenicus								
172	9.3 ± 0.3	5.6 ± 0.6	1.5 ± 0.8	2.5 ± 0.5	<1.0	2.1 ± 1.2	1.3 ± 1.1	10.7 ± 0.8
292	9.8 ± 0.6	5.6 ± 0.6	1.2 ± 0.4	4.1 ± 0.5	3.2 ± 0.8	3.1 ± 1.6	2.8 ± 0.8	9.8 ± 0.8
375	9.6 ± 0.7	7.4 ± 0.8	6.4 ± 1.2	3.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.8	1.2 ± 0	9.4 ± 0.6
446	10.4 ± 0.4	7.2 ± 1.2	5.8 ± 1.0	2.0 ± 0.4	1.6 ± 0.6	1.8 ± 0.6	1.0 ± 0.6	12.1 ± 1.8

[a] Part of experiment presented in Table 3. CFU are expressed as $\log_{10}$ per abscess and are the means ± standard deviations of specimens derived from six mice per group. −, Without gentamicin; +, with gentamicin (7.5 mg kg per day).

TABLE 6. Impacts of treatment with clindamycin and metronidazole alone and in combination with gentamicin on CFU in abscesses of mice infected with isolates of the *B. fragilis* group

Isolate	Mean ± SD $\log_{10}$ CFU after treatment with following drug dose (mg kg per day)[a]					
	None (untreated mice)	Clindamycin (40)		Metronidazole (50)		Gentamicin alone (7.5)
		−	+	−	+	
B. fragilis						
13	10.4 ± 0.5	7.2 ± 0.7	5.9 ± 0.8	4.3 ± 0.8	1.2 ± 1.0	10.4 ± 0.6
21	9.6 ± 0.8	5.2 ± 0.8	4.2 ± 1.2	3.3 ± 0.6	2.6 ± 1.2	10.1 ± 1.2
38	10.2 ± 1.2	6.4 ± 1.2	5.3 ± 0.8	2.6 ± 0.8	3.4 ± 1.4	9.2 ± 1.4
43	9.6 ± 1.4	4.2 ± 1.4	4.4 ± 0.6	4.4 ± 1.2	4.2 ± 0.8	8.8 ± 1.1
52	8.6 ± 2.0	5.2 ± 0.8	6.4 ± 1.2	5.5 ± 1.4	4.2 ± 0.6	9.6 ± 0.8
181	10.4 ± 1.6	4.6 ± 0.4	4.2 ± 1.0	3.7 ± 1.4	4.1 ± 0.4	10.2 ± 1.6
B. vulgatus						
200	9.8 ± 1.2	4.2 ± 0.6	3.6 ± 0.8	2.1 ± 0.8	3.0 ± 1.2	9.0 ± 0.7
60	10.2 ± 1.0	2.0 ± 1.2	1.7 ± 0.6	3.9 ± 0.7	3.9 ± 0.4	8.5 ± 0.7
583	9.6 ± 0.6	3.4 ± 0.8	5.0 ± 1.2	3.8 ± 1.2	3.4 ± 0.8	9.2 ± 0.8
B. ovatus						
22	10.6 ± 0.7	3.1 ± 0.8	2.5 ± 0.5	2.4 ± 0.5	3.1 ± 0.5	10.2 ± 0.5
105	9.6 ± 0.8	4.3 ± 1.2	4.2 ± 1.6	2.4 ± 0.8	2.4 ± 0.4	8.1 ± 0.8
234	10.2 ± 0.6	5.5 ± 1.4	5.4 ± 1.4	4.4 ± 1.2	3.6 ± 0.8	9.8 ± 0.5
B. thetaiotaomicron						
27	10.8 ± 0.3	2.0 ± 1.1	1.8 ± 0.8	1.3 ± 1.1	1.6 ± 0.8	10.9 ± 0.5
85	9.6 ± 0.4	6.4 ± 1.2	5.2 ± 1.4	3.3 ± 1.0	2.8 ± 1.0	10.2 ± 0.4
176	8.8 ± 0.8	6.6 ± 1.4	6.4 ± 1.2	2.1 ± 0.4	2.0 ± 0.8	9.4 ± 1.1

[a] Part of experiment presented in Table 4. CFU are expressed as $\log_{10}$ per abscess and are the means ± standard deviations of specimens derived from six mice per group. −, Without gentamicin; +, with gentamicin (7.5 mg kg per day).

melaninogenicus. Synergistic interaction between aminoglycosides and penicillins have been noted and studied with certain aerobic or facultative anaerobic organisms (17). For example, this combination was found to be effective in the treatment of enterococcal and staphylococcal diseases. It has been postulated that the penicillins, which inhibit cell wall synthesis, enhance the penetration of aminoglycosides, which are capable of interacting with the ribosomes. There is circumstantial evidence that such a mechanism may prevail in *B. melaninogenicus*. Bryan et al. (3) demonstrated that cell-free amino acid incorporation *B. fragilis* ribosomes was inhibited by gentamicin to about the same extent as with *Escherichia coli* ribosomes. Furthermore, there was no evidence of inactivation of the antibiotic by *B. fragilis* cell extracts. Whole cells of *B. fragilis*, however, did not show any time-dependent accumulation of the antibiotic. This failure was attributed to the lack of the proper electron transport system for the transport of the aminoglycoside. The mechanism by which penicillin presumably permits the transport of aminoglycosides in *Bacteroides* spp. has not been investigated.

TABLE 7. Concentration of antimicrobial agents in sera and abscesses of mice[a]

Antimicrobial agent (daily dose [mg kg])	Mean concn (μg ml) of drug (time after last administration)			
	Serum[b]		Abscess fluid (0.5 h)	
	0.5 h	8 h	*B. melaninogenicus*[c]	*B. fragilis*[c]
Penicillin G (100)	27.5 ± 8.2[d]	13.6 ± 3.1	34.0 ± 7.6	8.2 ± 2.1
Clindamycin (40)	8.9 ± 3.4	2.4 ± 0.8	14.6 ± 3.6	13.4 ± 4.2
Metronidazole (50)	28.6 ± 6.4	11.0 ± 2.6	12.2 ± 3.4	13.2 ± 3.6
Gentamicin (7.5)	5.4 ± 2.2	1.2 ± 0.4	3.8 ± 1.8	4.0 ± 2.0

[a] Determined 7 days after inoculation.
[b] *B. asaccharolyticus* 114.
[c] *B. fragilis* 13.
[d] Mean ± standard deviation of 10 mice in each group.

There is no obvious explanation for the in vitro and in vivo synergism between clindamycin and gentamicin against *B. melaninogenicus* and the less pronounced synergism between metronidazole and gentamicin against both *Bacteroides* groups. That increased gentamicin transport is also involved in these synergisms is an attractive hypothesis worth investigating.

Combinations of antibiotics are continually being studied in attempts to discover more effective therapy for serious infections. Combined therapy, in addition to its more obvious effects, might delay emergence of antimicrobial resistance or provide broad coverage for unidentified pathogens. Busch et al. (4) suggested that the combination of clindamycin and metronidazole might prove useful in the treatment of selected infections, such as endocarditis, septic thrombophlebitis, and osteomyelitis, in which *B. fragilis* is implicated as a single or primary pathogen. Although drawing clinical importance from our study, and in particular from results with the penicillin-gentamicin combination against *B. melaninogenicus*, is premature, the data here presented open the possibility of a new approach for the treatment of this infection.

ACKNOWLEDGMENTS

We gratefully acknowledge J. D. Gillmore and J. E. Perry for their excellent technical assistance, G. Pazzaglia for statistical analysis, C. H. Dorsey for electron microscopy, and Donna Boyle for secretarial assistance.

This work was supported in part by grant 68011 from the Upjohn Co., Kalamazoo, Mich.

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